0.22 G), and k is a mixture of equal parts of T and G. The bases shown in lower case at either end are spacers and are not incorporated into the cloned gene. One of the variegated oligo-nts and the primer are combined in equimolar amounts and annealed. The ds DNA is completed with all four (nt)TPs and Klenow fragment. The resulting dsDNA and RF pLG7 are cut with both Kpn I and Xho I, purified, mixed, and ligated. This ligation mixture goes through the process described in Sec. 15 in which we select a transformed clone that, when induced with IPTG, binds AHTrp or trp.

Other numbers of variegated codons could be used.

If none of these approaches produces a working chimeric protein, we may try a different signal sequence. If that doesn't work, we may try a different OSP in M13 because the structural data clearly indicate that BPTI could not be joined to the carboxy terminus. The next best OSP of M13 is the gene III protein because there is fusion data (SMIT85, CRUZ88).

Example 1, Part II

BPTI binds very tightly to trypsin (K_d = 6.0 x 10^{-14} M) and to anhydrotrypsin, so that these molecules are not preferred for optimizing the amount of BPTI to display on LG7 or the amount of affinity molecule to attach to the column. Tschesche et al. reported on the binding of several BPTI derivatives to various proteases: